PCT/GB2004/002743 WO 2005/001124

- 26 -

Claims.

A method of characterising a target base in a sample nucleic acid, which method comprises:

- contacting the sample nucleic acid with an (a) oligonucleotide primer under conditions which allow hybridisation of the oligonucleotide to the sample nucleic acid, said oligonucleotide primer being labelled with a fluorophore;
- contacting the sample nucleic acid with a (b) deoxynucleotide or dideoxynucleotide which is labelled with a fluorophore, under conditions which allow extension of the oligonucleotide primer through incorporation of the labelled nucleotide; and
- measuring the fluorescence emitted by one or both (c) of the fluorophores.
- A method according to claim 1, wherein one fluorophore can act as a donor and the other fluorophore can act as an acceptor.
- A method according to claim 1 or 2 wherein the oligonucleotide primer fluorophore acts as a donor and the nucleotide fluorophore acts as an acceptor.
- A method according to claim 1 or 2 wherein the 4. oligonucleotide primer fluorophore acts as an acceptor and the nucleotide fluorophore acts as a donor.
- 5. A method according to any one of claims 2 to 4 wherein fluorescence resonance energy transfer can take

WO 2005/001124 PCT/GB2004/002743

- 27 -

place between the donor and the acceptor fluorophore when the primer is extended by incorporation of the labelled nucleotide.

- 6. A method according to any one of claims 1 to 5 wherein step b) further comprises contacting the sample with a DNA polymerase and carrying out a thermo-cycling reaction.
- 7. A method according to any one of claims 1 to 6 wherein step c) comprises irradiating the sample nucleic acid and measuring the fluorescence emitted by one or both of the fluorophores.
- 8. A method according to any one of claims 1 to 7 wherein the fluorescence emitted by the fluorophore of the oligonucleotide primer is recorded.
- 9. A method according to any one of claims 1 to 8 wherein the fluorescence emitted by the fluorophore of the deoxynucleotide or dideoxynucleotide is recorded.
- 10. A method according to any one of claims 1 to 9 wherein the primer is designed such that the 3' end of the primer hybridises immediately upstream of the target base.
- 11. A method according to any one of claims 1 to 10 wherein the labelled nucleotide is a dideoxynucleotide.
- 12. A method according to any one of claims 1 to 11 wherein a plurality of target bases are characterised.
- 13. A method according to any one of claims 1 to 11 wherein only one species of labelled primer is used in

WO 2005/001124 PCT/GB2004/002743

step a) and only one species of labelled nucleotide is used in step b).

- 14. A method according to claim 12 wherein one species of labelled primer and a plurality of different species of labelled nucleotides are used.
- 15. A method according to claim 14 wherein each species of nucleotide is labelled with a different type of fluorophore.
- 16. A method according to claim 12 wherein a plurality of different species of labelled primers and one species of labelled nucleotide are used.
- 17. A method according to claim 16 wherein each species of primer is labelled with a different type of fluorophore.
- 18. A method according to any one of claims 1 to 17 wherein the fluorescence emission maxima of the two fluorophores are at least 15 nm apart.
- 19. A method according to claim 18 wherein the fluorescence emission maxima of the two fluorophores are at least 30 nm apart.
- 20. A method according to any one of claims 1 to 19 wherein the wavelength of the light used for irradiation is such that the light is only efficiently absorbed by the donor and direct excitation of the acceptor is negligible.
- 21. A kit for use in a method according to any one of claims 1 to 20, which kit comprises:
- a) an oligonucleotide primer labelled with a

WO 2005/001124 PCT/GB2004/002743

- 29 -

fluorophore;

- b) a deoxynucleotide or dideoxynucleotide labelled with a fluorophore; and optionally
- c) a polymerase